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Title: Honey bees and neonicotinoid-treated corn seed: contamination, exposure, and effects

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Abstract

Most corn (*Zea mays*) seeds planted in the US in recent years are coated with seed treatment products that contain two neonicotinoid insecticides, clothianidin and thiamethoxam. Fine dust particles of the seed treatment resulting from abrasion during planting can easily disperse through the landscape. Honey bee mortality incidents related to corn planting have been well-documented in North America and Europe. However, a clearer understanding of the route through which bees are exposed to these insecticides during corn planting is urgently needed to effectively mitigate the problem. We examined the presence of corn seed treatment insecticides in bee-collected pollen and increased honey bee mortality associated with corn planting, persistence of the insecticides inside honey bee colonies, and long-term growth of these colonies in central Ohio. We then constructed spatial models, based on empirical data of honey bee foraging and dispersion patterns of planter dust, and landscape compositions, to simulate hypothesized exposure routes via contamination of foraging resources and aerial exposure resulting from flight through localized dust plumes from planters and diffuse dust in the landscape over all resulting from widespread planting activity. Insecticide concentrations under different hypothesized exposure routes were then compared with the observed levels of contamination to evaluate these hypotheses.

For three years we consistently recorded elevated honey bee mortality and increased residues of clothianidin and thiamethoxam in bee-collected pollen during corn planting. However, we did not observe vital effects of the exposure on brood production, food storage, or winter survival of the colonies.

The observed level of exposure to clothianidin and thiamethoxam in pollen contamination was correlated with the area of corn planted within the foraging range of an apiary, supporting the hypothesis that the primary route of exposure is through a diffuse cloud of insecticide-laden dust generated when corn planting activity is most intense. Absence of correlation between the observed level of exposure and predicted contamination in foraging resources suggested that weed control or management of floral resources in field margins will not be effective as a mitigation approach and could starve bees and other pollinators of floral resources. Instead, future work should focus on reducing the initial release of insecticide-laden seed treatment

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particles from corn seeds.

Keywords:

Introduction

It is estimated that over 79% of corn (*Zea mays*) hectares in the United States are grown from seed treated with neonicotinoid insecticides (Douglas and Tooker 2015). The predominant neonicotinoids used in corn seed treatments are clothianidin (Poncho®) and thiamethoxam (Cruiser®) at rates between 0.25 and 1.25 mg per seed (Douglas and Tooker 2015). Assuming a seeding rate of 54,340 – 81,510 seeds per hectare (Thomison 2015), up to 100 g/hectare of insecticide active ingredients are applied to sown fields each year. These broad spectrum insecticides are highly toxic to many non-target insects, including honey bees (*Apis mellifera*), to which they are lethal in nanogram quantities (as low as 0.003 µg/bee for oral LD_{50} and 0.02 µg/bee for contact LD_{50} over 48 hr) (Decourtye and Devillers 2010, Laurino et al. 2013).

A link between observations of honey bee mortality and the planting of neonicotinoid-treated corn seeds was suspected as early as the late 1990s when researchers in Italy noted a rise in colony damage reports coinciding with spring corn planting (Bortolotti et al. 2009). In subsequent years, similar patterns of honey bee mortality were observed in Italy (Schnier et al. 2003, Greatti et al. 2006, Bortolotti et al. 2009), France (Giffard and Dupont 2009), and Slovenia (Alix et al. 2009, van der Geest 2012, Žabar et al. 2012). In 2008, a large-scale bee kill in Germany and neighboring parts of France was attributed to the planting of neonicotinoid-treated corn after an extensive investigation found neonicotinoid residues in dead bees, pollen stores, and plant samples collected from the affected area (Forster 2009, Nikolakis et al. 2009, Pistorius et al. 2009, Chauzat et al. 2010). Since then, additional incidents of honey bee mortality during corn planting have been reported in Slovenia and neighboring Hungary (van der Geest 2012), the United States (Krupke et al. 2012); L. Keller, personal communication, 2016) and Canada (Health Canada 2013).

While these reports clearly establish a link between the planting of neonicotinoid-treated corn and honey bee mortality, the mechanistic basis for this relationship is difficult to ascertain, due in part to the multiplicity of potential exposure routes. During the planting process, seed treatment material containing insecticides sloughs off the seed surface in small particles that disperse in the environment (Figure 1). Foraging bees may encounter these particles in the course of foraging. Exposure to seed treatment insecticides has been hypothesized to occur through a several routes, including physical contact with dust deposited on the surface of flowers (Krupke et al. 2012), nectar and pollen contamination via uptake from the soil (Long and Krupke 2016), contamination of surface water (Samson-Robert et al. 2014; Schaafsma et al. 2015), contamination of guttation fluids (Tapparo et al. 2011), and direct contact between aerial dust

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and flying bees (Girolami et al. 2012). Soil containing insecticides left over from previous years of planting may also become airborne during planting and contribute to bee exposure during this period (Forero et al. 2017). While each of these routes may contribute to honey bee exposure to some degree, identifying the route or routes of most importance is critical for appropriate mitigation.

To better understand the association between corn planting, neonicotinoid residues in honey bee-collected pollen, and honey bee mortality, we conducted three years of field work (2013 - 2015) in the state of Ohio, USA. We measured worker mortality and sampled bee-collected pollen to determine the concentrations of corn seed treatment insecticides in pollen prior to, during, and after corn planting. Additionally, in 2015 we monitored hive development (adult bee population, brood production, and pollen and honey storage) at 10 locations across a gradient of corn-planting intensity throughout the summer and the following year.

Using these data, we constructed statistical models to evaluate the predictions of several proposed routes of exposure that could account for observed patterns of adult bee mortality and seed treatment insecticide residues in bee-collected pollen. The models partitioned contamination into different landscape components weighted by the probability of being encountered by foraging honey bees to estimate the relative magnitude of neonicotinoid exposure. according to hypothesized routes of exposure and predicted exposure according to these hypotheses were compared to the observed clothianidin and thiamethoxam residues in bee-collected pollen to identify the hypotheses that best explains the level of contamination observed in field.

Results

Worker bee mortality

Increased numbers of dead bees at hive entrances were consistently observed around the time when corn was being planted. The daily worker mortality index ("mortality" hereafter), calculated from dead bees counted at the hive entrance and standardized by month-average mortality of the colony (see **Eq. 1** in Methods), was significantly and consistently higher during corn planting than the non-planting periods for the same colonies for all years (2-tailed paired t-test comparing mortality of the same colony averaged during planting vs. non-planting periods; 2013: $t = 2.79$, $df = 11$, $P = 0.0175$; 2014: $t = 3.02$, $df = 23$, $P = 0.0061$; 2015: $t = 9.12$, $df = 37$, $P < 0.0001$) (**Fig. 2a**). The mean number of dead bees at colonies was consistently greater than zero for all years (**Fig. 2a**), indicating elevated mortality is associated with corn planting.

Commented [1]: You mean the mortality index, not the raw dead bee counts, right? Since dead bee counts will, of course, always be > 0.

Pollen contamination

Our data of insecticide-contaminated pollen in relation to the timing and intensity of corn

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planting all indicated that corn seed treatment dust was the main source of neonicotinoids found in pollen. Clothianidin and thiamethoxam, the insecticidal ingredients in corn seed treatment, were consistently the most abundant insecticides detected in bee-collected pollen for three years. Detection of clothianidin and thiamethoxam occurred more frequently (Fisher's Exact Test, $P < 0.0001$) and at higher concentrations (**Table 1, Fig. 2b**) in pollen sampled during planting than non-planting periods. Other neonicotinoid insecticides including imidacloprid, nitenpyram, dinotefuran, and thiacloprid were also found in some pollen samples (**Supplemental Material S2**). In pollen collected during corn planting, the levels of clothianidin and thiamethoxam were correlated with the percent area of cornfield with in 2 km radius from the apiaries (STATS, Jeff's analysis). No correlation with cornfield area was detected in clothianidin or thiamethoxam outside the planting period, or in any of the other neonicotinoid compounds at any time (STATS, Jeff's analysis).

Relation of pollen contamination and worker mortality

Worker mortality was significantly higher on days when the detectable clothianidin and thiamethoxam residues were present in pollen samples than mortality of the same colonies when the residues were absent (2-tailed paired t-test; 2013: $t = 2.13$, $df = 11$, $P = 0.0565$; 2014: $t = 3.82$, $df = 23$, $P = 0.0009$; 2015: $t = 8.13$, $df = 33$, $P < 0.0001$) (**Fig. 2c**)

To determine if there was any interactive effect of the timing of planting and corn intensity in the landscape, we further tested for correlations between the number of dead bees and clothianidin and thiamethoxam concentrations in pollen for each apiary in 2015. Positive correlations between clothianidin and thiamethoxam concentrations and mortality were more likely at sites with more intense corn agriculture (> 30%) in the surrounding landscape (**Table 2**).

Insecticide residues in in-hive samples

Samples of bee bread, honey, larvae, and nurse bees were collected from two colonies at seven apiaries (DS, SC, IB, HR, TV, BG, MM, corn area ranging 1 - 39%) four times (pre-planting, during planting, post-planting, and two weeks post-planting). Clothianidin and thiamethoxam concentrations in honey and bee bread were low at most sites before planting and showed no correlation with concentrations (< xx ng/g, N = xx, **Supplemental Material S2**). The levels and increased once corn-planting had begun and to a higher level at apiaries surrounded by more corn fields.

There was no significant correlations between levels of clothianidin and thiamethoxam in pollen (cumulative concentration [[[Recal. stats with average]]]] during corn planting, May 2 - 8) and any of the in-hive samples collected before the peak planting period. During corn-planting, an emerging trend of correlation was observed between insecticide concentrations in honey and pollen ($r = 0.72$, $P = 0.0680$). Additionally, increased levels of clothianidin and thiamethoxam were detected in honey and bee bread samples, of which the insecticide concentrations were

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positively correlated with each other ($r = 0.75$, $P = 0.052$). Insecticide concentrations in bee-collected pollen were significantly correlated with honey ($r = 0.71$, $P = 0.07$) and bee bread ($r = 0.79$, $P = 0.0358$) immediately after the peak corn-planting period, indicating that the insecticides were deposited inside the hives along with stored food. Clothianidin and thiamethoxam concentrations in bee bread and honey were also significantly correlated ($r = 0.91$, $P = 0.0045$) for samples collected immediately after planting.
No significant correlation was found between insecticide concentrations in pollen and any of the in-hive samples two weeks after the peak planting period, suggesting the insecticides that had entered the hives had been consumed by bees or diluted at various rates.

Out of the 28 larval samples, detectable levels of clothianidin and thiamethoxam were only found in one sample collected on May 2 and another collected on May 5. Therefore, no statistical analysis was performed on the larval samples.

Closed nuc experiments

To test the direct association between corn seed treatment insecticides detected in pollen and worker mortality, we conducted a semi-field experiment where small honey bee colonies, enclosed in a controlled environment, were fed with pollen collected from our study apiaries that were contaminated with the observed range of clothianidin and thiamethoxam. A sudden increase in worker mortality was recorded within the first four days of the enclosure and the number of dead bees was positively correlated with the concentration of clothianidin and thiamethoxam in pollen fed to the bees (Pearson's $r = 0.79$, $P = 0.0001$; Fig. 4). This result confirmed the causal link between worker mortality and exposure to corn seed treatment insecticides.

Post-planting colony development.

To address the question of whether greater neonic exposure in May was linked to long term consequences in colony growth, we calculated relative changes in the six hive health variables (adult bees, stored pollen, stored nectar, open brood, and capped brood, measured by frame area) at four time points (April, May, June, and August). Greater exposure to neonics in May was associated with a reduction in the relative population growth of the hive (as measured by area of bees and seams of bees) over the earliest time interval (late April to late May) (area of bees: $t = -3.61$, $P = 0.01$; seams of bees: $t = -2.50$, $P = 0.04$). However, there may have been a recovery in the second time interval (late May to late June), as hives exposed to greater neonic levels in May had a larger increase in bee population during this time (for seams of bees only; $t = 2.47$, $P = 0.04$). Finally, in the third time interval (June to August), hives located in areas where neonic concentrations were highest in May had a greater increase in pollen area ($t = 3.35$, $P = 0.01$). This could reflect the increase in foraging resource in areas with more agricultural lands, as cornfield area

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was also positively correlated with the relative change in area for both pollen (Pearson's r = 0.79, P = 0.007) and nectar (r = 0.68, P = 0.03) during this time. This observation may be associated with food sources such as clover and other summer wildflowers that thrive in roadsides and field margins, and with the flowering of soybean, which is often planted in rotation with corn (Sponsler and Johnson 2015, Sponsler et al. 2017).

Of the 38 colonies monitored, one colony died in late summer and three were relocated to another location by the beekeeper and were excluded from overwinter monitoring. Therefore, a total of 34 colonies were prepared for overwintering at the end of September 2015. Thirty one of the 34 colonies (91%) were alive at the end of March, 2016, although one of the surviving colonies was queenless and had developed laying-workers. No significant correlation was observed between overwinter survival and the level of corn seed treatment insecticides in pollen or percent corn area in the surrounding landscape across the 10 apiaries (Spearman's rank correlation tests, P > 0.36 for all tests).

Modeling exposure routes

Spatial models that partitioned partitioned contamination into different landscape components and weighed by the probability of being encountered by foraging honey bees were constructed to evaluate several hypothetical routes of exposure that might account for the by observed patterns exposure, measured as clothiandin and thiamethoxax`m concentration in bee-collected pollen sampled in 2015. Observed exposure significantly correlated with exposure indices predicted by the **localized plume** (**Pearson's r = 0.65, P = 0.0407**) and **diffuse cloud** (**r = 0.75, P = 0.0131**) models on the day (May 5, 2015) when corn-planting activity was most widespread throughout the study region. No significant correlation were found between observed exposure and exposures predicted by any of the floral contamination models.

Discussion

Mortality, exposure, and corn planting

Key message 1: Clear association between worker mortality and seed dust exposure (short term), evidenced by the link between pollen contamination, dead bees, and timing of planting.

For three years, we consistently observed elevated mortality in adult honey bee workers during corn planting. This pattern of mortality coincided with our finding that clothianidin and thiamethoxam, the insecticidal component in corn seed treatment, were detected more frequently and at higher concentrations in pollen collected by honey bees during corn planting. Additionally, our study showed that seed treatment insecticides can consistently be detected on

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surfaces both within and adjacent to cornfields during planting, indicating that the release of seed treatment particles during corn planting is ubiquitous and that released particles are subject to aerial transport, in agreement with previous studies (Krupke et al. 2012, Schaafsma et al. 2015).

Together, these lines of evidence strongly indicate a causal connection between elevated honey bee mortality and seed treatment insecticides emitted during planting. This conclusion is further corroborated by recent work in Italy, where reports of honey bee mortality during corn planting have decreased significantly since the suspension of neonicotinoid seed treatments in corn (Sgolastra et al. 2017). Nevertheless, the level of mortality observed in our study was not sufficient to cause detectable long-term impacts on colony health. To the contrary, corn area was associated with more pollen and honey accumulation by colonies later in the summer, suggesting that the high floral abundance of the agricultural landscape (Sponsler and Johnson 2015, Sponsler et al. 2017) may have ultimately overcome the losses sustained due to insecticide exposure during corn planting.

One inconsistency must be considered, though. The concentrations of seed treatment insecticides detected in bulk pollen samples (range) were well below those that would be expected to cause acute mortality. Based on a range of acute oral LD50s for adult workers of 1.11 - 6.76 ng/bee (Laurino et al. 2013) and pollen consumption of 6.5 mg/bee/day(Rortais et al. 2005) substantial mortality would be expected at concentrations greater than 171 µg/kg in pollen. Both of these could be explained by the fact that insecticide residues measured in bulk pollen samples may not meaningfully reflect doses received by individual bees (Sponsler and Johnson 2017). For example, a bulk pollen residue of 20 µg/kg clothianidin could reflect a uniform distribution of insecticide such that every bee consuming the pollen would be exposed to a concentration of 20 µg/kg, or it could reflect one or a few "needles in the haystack", highly concentrated pollen pellets masked by the low contamination of the rest of the sample. These two distributions of insecticide could result in very different mortality rates, obscuring the correlation between mortality and bulk insecticide concentration and, in the case of the latter, creating a situation in which mortality could occur despite an ostensibly innocuous bulk concentration. Consistent with this interpretation, the pattern of insecticide deposition we observed in our study of seed treatment drift would predict a highly skewed distribution of contamination in which the relatively few flowers within corn fields would receive a level of contamination several times higher than that received by the majority of flowers outside of corn fields.

Post-planting colony development

Key message 2: No long-term, colony-level impact: (1) residues that entered hives dissipated ~2wk after exposure (2) no correlation found between exposure and hive parameters in summer

Although we found strong evidence that connected the surge in honey bee worker mortality with

the exposure to corn seed treatment insecticides emitted as planter dust during planting, we did not detect strong long-term impacts of the exposure on colony growth or winter survival. The lack of long-term effect at the colony level may be explained by the dynamics of clothianidine and thiamethoxam once these compounds had entered the hives. High levels of clothianidine and thiamethoxam were detected in bee bread and honey sampled immediately after the major corn-planting period, and were correlated with the levels of the same compounds in bee-collected pollen sampled during the planting period. However, by the second week after the planting period, insecticide residues in bee bread and honey decline to the pre-planting level for most cases. Contaminations in food stored inside the hives may have either been consumed or removed by workers, or diluted to a non-lethal level as the worker bees mixed in clean pollens collected post-planting.

However, the sub-lethal effects of the exposure remain unclear and require further investigation. The sudden loss of foragers occurred at the critical time when colonies are building up workers for early-summer honey productivity and small colonies may not be able to recover from the loss (Khoury et al. 2013). The exposure could potentially affect queen quality as the planting season coincides with the time when colonies make new queens (swarm season) (Tsvetkov et al. 2017). Clothianidine and thiamethoxam can also affect honey bee's immunity against viral diseases (Di Prisco et al. 2013) or reduce survival when colonies are under nutritional stress (Tosi et al. 2017). [[[check references]]]]

Model interpretation/implication for mitigation strategies

Key message 3: Model interpretation: exposure (residues in pollen) was predicted by localized aerial plume and ubiquitous aerial exposure during planting, but not by the amount and location of foraging resources in the landscape.

The spatial models of exposure routes revealed that the observed pattern of exposure can be best explained by aerial contact with localized plume or diffuse cloud of planter dust emitted during planting. This suggests that the contact with insecticide-laden dust particles as foraging bees fly through the landscape is the primary mechanism of the exposure. Tapparo et al. (2012) show that extremely high levels of exposure are possible when flying bees intersect directly with the discrete plume of seed treatment particles emitted by a running planter, but these direct intersection events must be extremely rare given the small size of the roughly 40 m radius of the planter plume (Girolami et al. 2013) compared to the massive area foraged by a honey bee colony.

Although the models did not suggest contaminated floral resources as an explanatory factor of the observed exposure, some of the residues in bee-collected pollen may come from dust landing on flowers or residue inside pollen as a result of systemic uptake of the insecticides in soil.

We found no evidence suggesting that removal or manipulation of foraging habitats (in fields being planted or within the planter drift zone) may effectively mitigate honey bee exposure to seed treatment neonicotinoids. On the contrary, a landscape with little foraging habitats might force bees to fly longer distance to collect food, and pick up more dust particles in flight. At site B (FSR), the site where the highest mortality and insecticide contamination were recorded in this study also had the lowest amount (near zero) of field weeds. A possible explanation is suggested by the dance language analysis (**Supplemental Material S3**). Of the four apiaries where dance analysis was performed, B was peculiar in that peak visitation probability occurred about 1.5 km from the hive, while at all other apiaries peak visitation probability occurred in the immediate vicinity of the hive (**Supplemental Material S3, Figure S3.xx**). This indicates that forage was relatively scarce, perhaps due to the scarcity of in-field weeds, forcing bees to forage farther from the hive to find suitable resources. This dilation of the foraging radius would greatly increase both the amount of time spent in flight and the amount of airspace traversed by foraging bees. Since aerial contact exposure should be proportional to flight time and/or the amount of airspace traversed, it is reasonable to think that aerial contact exposure may have been exceptionally high at B.

Conclusion

Our study confirms that seed treatment insecticides are released during corn planting, that these insecticides contaminate pollen collected by bees, and that honey bee colonies experience elevated adult mortality due to seed treatment exposure. The level of mortality we observed, however, was not sufficient to cause detectable long-term effects on colony health. Our results support the hypothesis that the exposure of honey bees to seed treatment neonicotinoids during corn planting is driven primarily by the aerial contact of planter dust particles by foraging bees.

Therefore, mitigation approaches involving removal of floral resources in or adjacent to cornfields prior to planting may not be effective or may even exacerbate exposure through aerial contact by forcing bees to increase their foraging time. Recommended solution would be to reduce the use of seed treatment products as an insurance policy and improve equipment designs to minimize the dispersion of planter dust in circumstances where seed treatments are unavoidable.

Methods

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Study sites

A total of 13 apiaries located throughout the corn-growing region of central Ohio were monitored prior to, during, and after corn-sowing from late-April to end of May in 2013 (3 apiaries), 2014 (6 apiaries) and 2015 (10 apiaries). Four apiaries were studied in multiple years (Supplemental Material S2). Apiaries were located at least 4 km from each other and were selected to represent a range of agricultural intensity, including one suburban apiary in 2015, with minimal corn agriculture within foraging range. Apiaries consisted of between 4 - 20 colonies. Two to four healthy, actively foraging colonies, varying in sizes and queen ages, were monitored for worker mortality (see Supplemental Material S2 for colony information). All colonies were housed in eight- or ten-framed Langstroth hives.

The timing of corn sowing activity was identified through direct observation of planting activity near apiary sites and communication with farmers, and were in line with state-wide agricultural statistics for each year (USDA NASS). The bulk of sowing activity in this region occurred between May 5 – 16 in 2013, May 5 – 10 in 2014, and May 2 – 8 in 2015. Less intensive corn planting continued beyond this period in all years, but was particularly drawn out in 2014 when high rainfall resulted in planting and re-planting through the end of May.

Landscape characterization

The landscape surrounding each apiary was characterized within a 2-km radius around the apiary. Visual ground-truthing supplemented by satellite imagery (Google OpenLayers), was used to classify landscapes into crop field, forest, treeline, herbaceous strips in field margins and roadsides, and residential lots. Crop type was determined by a second visual inspection in early summer and the USDA crop data layer (USDA-NASS-RDD). All landscape data were analyzed and visualized using QGIS software (QGIS Development Team 2015). Immediately prior to corn planting, each agricultural field was visually assessed to determine the abundance of in-field flowering weeds and scored as "abundant", "sparse", or "absent".

Apiaries in 2013 and 2014 were surrounded by a high proportion of corn fields within 2 km of the apiaries, ranging from 31 – 45% corn in 2013 and 21 – 51 % in 2014. In 2015, a wider gradient of corn area was in the foraging range, 0 - 49% corn.

Worker bee mortality

Under-basket style dead bee traps (102x51x15 cm; (Human et al. 2013) were placed in front of each colonies being monitored. Dead bees in traps were counted and removed every 2 – 4 days, starting in late April each year, approximately one week before corn planting, until 1 - 2 weeks after planting activities had ceased. Averaged number of dead bees per day was calculated for each sampling interval as the number of dead bees in the trap divided by number of days elapsed since the last sampling. Because large colonies eject more dead bees than small colonies, we

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converted the daily dead bees count for each hive to a mortality index, denoted by M_i, using the following formula:

$$M_i = (N_i - N_a)/N_a \qquad \textbf{Eq. 1}$$

where
N_i is the number of dead bees per day on a given date i
N_a the average number of bees per day for a colony collected over the entire sampling period

The standardized mortality, M_i is the difference between daily mortality and the overall mortality for a given colony over the sampling period. If mortality is consistent throughout the sampling period, then M_i is expected to be zero. To compare bee mortality between planting and non-planting periods, we took the *means* of M_i values for each hive during planting and non-planting periods respectively. Each hive has two values representing mortality during the planting and non-planting periods respectively. Paired-sample t-tests were performed to compare mean mortality of the same hives during planting vs. non-planting periods. A separate analysis was performed for each year.

Sampling and pesticide screening

Incoming corbicular pollen was collected for pesticide screening from bees returning to two strong colonies in each apiary using bottom-mounted pollen traps (Sundance I, Ross Rounds, Inc.). Pollen was collected every 2-4 days, pooled by site and date and stored at -20 °C until further analyses. In 2015 additional samples were collected for pesticide screening, including: dead bees from dead bee traps and in-hive samples of bee bread, honey, larvae, and live nurse bees. In-hive samples were collected from two queen-right, overwintered colonies at seven apiaries (DS, SC, BG, HR, TV, MM, Supplemental Materials S2). The in-hive samples were collected during four sampling periods: before planting (April 27 - 30), during planting (May 5 - 7), immediately after planting (May 12 - 13), and two weeks after planting (May 20 - 22). Honey and bee bread were collected from uncapped cells peripheral to the brood area where bees were actively depositing food. Nurse bees on the brood area and 25 - 30 late-stage larvae in open cells were collected from each hive. In-hive samples were pooled by apiaries and stored at -20 °C until further analyses.

Five grams of pollen from each site and sampling date were extracted using a modified QuEChERS protocol for 2013 - 2014 samples (Camino-Sanchez et al. 2010). Samples from 2015 were extracted following a method by Yáñez et al. (2014) except ethyl acetate was used instead of dichloromethane. In all years extracts were analyzed for neonicotinoid insecticides (clothianidin, thiamethoxam and imidacloprid) using liquid chromatography tandem mass spectrometry (LC-MS-MS) methods. Analysis was performed by the USDA-AMS lab in Gastonia, North Carolina (2013 - 2014 samples) and EPA National Exposure Research

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Laboratory in Athens, Georgia (2015 samples). All residues were reported as mass-mass concentration (µg/kg).

Statistical analyses

Mortality and pollen contamination associated with planting:
At the colony level, paired t-tests were performed to compare mortality with in averaged during planting vs. non-planting periods within the same colonies. The same tests were also performed to compare mortality of the same colonies recorded on dates with vs. without detectable clothianidin and thiamethoxam residues in pollen.
Non-parametric Fisher's Exact tests were performed to compare the frequency of positive detection of clothianidin and thiamethoxam residues in pollen collected during planting vs. non-planting periods. T-test assuming unequal variance was performed to compare clothianidin and thiamethoxam concentrations in pollen collected at each apiary during planting vs. non-planting periods.

Association between mortality and pollen contamination:
Non-parametric Fisher's Exact Tests were performed to test the hypothesis that mortality was higher on dates when detectable levels of clothianidin and thiamethoxam were present in pollen. Pairwise correlation between mortality and clothianidin and thiamethoxam concentration in pollen collected on the same sampling dates was evaluated for each site.

In-hive samples: Pairwise correlations between the cumulative clothianidin and thiamethoxam concentrations in corbicular pollen sampled during the peak planting period (May 2 - 8) and each of the in-hive samples from corresponding apiaries were analyzed separately for each sampling date.

Closed colony experiments:
Closed nuc experiment, semi-field setup to test the link between insecticides in pollen and worker mortality.

- four trials, ~ one frame of nurse bees and an egg-laying queen that are 3 - 5 weeks post-mating. Each colony consisted of two frames with a small amount of capped brood (to stabilize the colony cohesion). The equipments were weighed prior to adding bees and again after bees were shaken into the box to obtain the net weight bees per colony. Pollen and dead bee samples were collected every 4 days for 12 days. Fresh sugar syrup (50% w/w) was provided liberally and replenished every 3 - 4 days.
- Colonies with < 100 grams of bees were excluded from the analysis.
- Treatments: corbicular pollen trapped in the field study, selected based on two criteria: (1) to simulate exposure to a wide range of CT concentration via pollen (2) availability of pollen. Each trial also contained a "positive control" treatment of which a relative clean pollen (< 10 ppb)

- Statistical Analysis: Pearson's correlation between dead bees and neonicotinoid concentrations for each sampling points (Day 4, 8, & 12)

Post-planting colony growth

To address the question of whether exposure to corn seed treatment insecticides in May is linked to long-term consequences in colony growth, we tracked the colonies from April 2015 through February 2016. Four detailed colony inspections were performed using a modified Liebfelder method (Delaplane et al. 2013) on April 28 – 30 (before planting), May 20 – 22 (after planting), June 19 - 24, and August 14 - 19. During the inspection each frame was removed from the monitored colonies to record the area of coverage with the following components: adult bees, brood (open and capped), pollen, and honey. Additionally, the total adult bee population was estimated by looking up and down frame spaces to estimate "seams" of bees. All colonies were managed using standard beekeeping practices. *Varroa* mites were controlled by applying formic acid (Mite Away Quick Strip, NOD Apiary Products, Ltd. Frankford, ON, Canada) in June and vaporized oxalic acid in November. Plain baker's fondant (Dawn Food Products, Inc., Jackson, MI, USA) and Dadant AP23 winter patties (Dandant & Sons Inc., Hamilton, IL, USA) were fed to the colonies, as needed, through the winter.

We examined whether the relative change in each colony variable through time was associated with neonic concentrations measured in pollen in May. Relative change for each variable was calculated as:

$$\square\square\square\square\square\square\square\square\ \square\square\square\square\square\square\ (\%) = \frac{\square\square\square\square\square - \square\square\square\square\square\square\square}{\square\square\square\square\square\square\square} * 100$$

We considered each interval between inspection dates, as well as the interval between the first and last inspections. To determine whether neonic loads in pollen were significantly associated with colony growth through time, we constructed linear regression models with relative change as the response and mean clothianidin and thiamethoxam concentrations in pollen in May as the predictor. We also included the relative change in colony pollen coverage over the same time interval as a covariate, to account for the potential that the negative effects of neonic exposure could be partially offset by the positive effects increased food supply. If the pollen change covariate was not a significant predictor, we dropped the term and refit the model.

Spatial modeling of routes of seed treatment dust exposure

With the premise that exposure requires the intersection of foraging bees with environmental contamination, we developed spatial models to evaluate possible routes that connect honey bees to corn seed treatment insecticides emitted as planter dust. The spatial models included

landscape characterization and empirical data of honey bee foraging range and the dispersion patterns of planter dust particles as predictor variables.
Details about data collection and model construction are described in **Supplemental Materials S3**. Briefly, we estimated honey bee foraging range by analyzing waggle dances, an unique behavior performed by honey bees to communicate resource locations with their nest mates (add References- von Frisch 1967, Couvillon et al. 20xx). We analyzed dances performed by returning foragers in glass-walled observation hives installed at four apiaries (FSR, HR, MB, and MO) during May xx – xx, 2015. Using the waggle dance data, we then created a two-dimensional spatial model (namely the foraging model) which predicted the probability of a 1 m^2 patch intersects with the foraging path of bees as a function of distance from the bees home hive.

Dispersion pattern of planter dust particles was examined in a separate study in 2014 and 2015. Using a modified dosimeter protocol (Krupke et al. 2012), we quantified corn seed treatment insecticides at various distances (0 – 100 m) from a total of [[[[how many?]]]] cornfields where planting trials were conducted (see Supplemental Material S3 for additional information). A piecewise model that estimates the surface concentration (ng a.i. per cm^2) of corn seed treatment insecticides as a function of distance from the test field was generated (Eq. XX in S3) and incorporated with the 2015 cornfield location, resulting in a raster layer of insecticide concentration in the surrounding landscape for each apiary.

The amount of corn seed treatment insecticides that may be encountered by foraging bees in the landscape can be estimated by multiplying its pesticide concentration by the probability that foraging honey bees will traverse it (aerial contact) or collect food from it (floral contamination). An index of predicted exposure in a contaminated landscape, then, can be defined as

$$\text{Predicted exposure} = \sum_{1}^{n} C_i P_i$$

where C_i is the concentration of insecticide in the *i-th* patch and P_i is the probability that foraging honey bees will traverse or collect food from this patch.

Therefore, the predicted exposure is the cumulative product of contamination and visitation probability for a given landscape and should be understood as an effectively unitless index. We can then estimate predicted exposure for different hypothetical scenarios that may explain the variation in corn seed treatment insecticides detected in pollen (which is used here as a proxy for the observed exposure)

Floral contamination:

(a) in-field settling: The observed exposure (*OE*) during corn planting is driven by the settling of seed treatment particles on flowering weeds in cornfields. In this scenario, exposure can be predicted by: *C(w)i represents insecticide concentration in the ith patch of cornfield with abundant weeds*

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$$\text{[illegible]} \sim \text{[illegible]}_{\text{[illegible]}} = \sum_{0}^{\text{[illegible]}} \text{[illegible]}_{(\text{[illegible]})\text{[illegible]}} \text{[illegible]}_{\text{[illegible]}}$$

(b) Off-field drift: If the observed exposure during corn planting is driven principally by off-field drift, then this should be a function of *drift exposure*, calculated as described above using *drift exposure raster*. or foragable habitat subject to drifting seed treatment neonicotinoids . D_i is the foragable habitats such as roadside vegetation and other natural or semi-natural habitat outside cornfields. C(d)i represents insecticide concentration in the ith patch of forabable habitat outside cornfields

$$\text{[illegible]} \sim \text{[illegible]}_{\text{[illegible]}} = \sum_{0}^{\text{[illegible]}} \text{[illegible]}_{(\text{[illegible]})\text{[illegible]}} \text{[illegible]}_{\text{[illegible]}}$$

(c) Combination of in-field settling and off-field drift (contamination of foraging resources in or near corn fields): If the observed exposure during corn planting is driven by total floral contamination–both weedy corn fields and off-field drift–then this should be functions of

$$\boldsymbol{OE \sim PE_{forage} = PE_{weedyCorn} + PE_{drift}}$$

Aerial contact:

(d) discrete plume: If observed exposure is driven principally by aerial contact with the discrete plume expelled by a running planter, then observed exposure should depend on the degree to which honey bee foragers traverse corn fields, regardless of whether they are weedy. This predicts that observed exposure should be functions of: C(c)i is represents insecticide concentration in the ith cornfield regardless of the field's foragability.

$$\text{[illegible]} \sim \text{[illegible]}_{\text{[illegible]}} = \sum_{0}^{\text{[illegible]}} \text{[illegible]}_{(\text{[illegible]})\text{[illegible]}} \text{[illegible]}_{\text{[illegible]}}$$

(e) diffuse cloud: If observed exposure is driven principally by aerial contact with a diffuse cloud of seed treatment particles, then this process should be largely independent of the exact locations and extents of corn fields. Instead, the amount of seed treatment particles in the air space over an area would depend simply on the total amount of corn planted in the region. Thus, observed exposure should be a function of total corn area ($Area_{Corn}$), regardless of weediness and visitation probability.

$$\boldsymbol{OE \sim Area_{corn}}$$

Modeling testing:

To test the prediction power of the models, the observed exposure (approximated as clothianidine and thiamethoxam concentration in pollen) was plotted against the predicted exposure index and a Pearson's correlation coefficient was calculated for each model. A separate evaluation for each model was performed for each sampling date. One apiary (SC) was excluded from all analyses involving weedy corn risk because a large proportion (40%) of corn fields at that apiary could not be accessed for bloom level ground-truthing. All analyses were performed

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in R (R Core Team 2015)

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Table 1. Clothianidin & thiamethoxam in pollen

Frequency of positive detections (Det./n, n = total number of samples) of clothianidin and thiamethoxam residues, and range of concentration when detected, in bee-collected pollen. T-tests assuming unequal variance were performed to compare the means of planting vs. non-planting periods.

		Det./n, range (µg/kg)		Mean±se (µg/kg)		
Year	Residues	Planting	Non-planting	Planting	Non-planting	t-test
2013	Clothianidin	12/12 (100%) 4.8 – 35.5	2/18 (11%) 3.9 – 6.9	16.63 ± 2.96	0.57 ± 0.40	t = 5.38, df = 11 **P = 0.0002**
	Thiamethoxam	8/12 (67%) 1.6 – 9.1	1/18 (6%) 2.2	3.67 ± 1.01	0.12 ± 0.12	t = 3.50, df =11 **P = 0.0048**
	Total	12/12 (100%) 4.8 – 44.6	3/18 (17%) 2.2 – 6.3	20.3 ± 3.75	0.69 ± 0.41	t = 5.19, df = 11 **P = 0.0003**
2014	Clothianidin	5/18 (28%) 12.0 – 18.4	0/55 (0%) N/A	4.15 ± 1.64	0	t = 2.53, df = 17 **P = 0.0219**
	Thiamethoxam	5/18 (28%) 5.6 – 9.3	0/55 (0%) N/A	1.94 ± 0.78	0	t = 2.49, df = 17 **P = 0.0233**
	Total	8/18 (44%) 5.6 – 21.1	0/55 (0%) N/A	6.09 ± 1.89	0	t = 3.24, df = 17 **P = 0.0048**
2015	Clothianidin	30/30 (100%) 2.2 – 91.9	33/60 (55%) 1.2 – 19.5	17.18 ± 3.43	2.89 ± 0.55	t = 4.11, df = 31 **P = 0.0001**
	Thiamethoxam	27/30 (100%) 1.4 – 46.5	33/60 (55%) 1.1 – 14.2	7.43 ± 1.79	2.20 ± 0.44	t = 2.83, df = 33 **P = 0.0039**
	Total	30/30 (100%) 3.6 – 138.4	41/60 (68%) 1.2 – 29.9	24.61± 4.87	5.09 ± 0.85	t = 3.95, df = 31 **P = 0.0002**

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Table 2. Interactive effect of site & pollen insecticide concentrations on mortality
Summary of Pearson's correlation test between worker mortality and clothianidin and thiamethoxam concentrations in bee-collected pollen for the 2015 sites. Sites are presented in the order of corn area (in %) within a 2 km radius centering the apiary.

Site	% corn	r	P
DS	1	0.5358	0.1371
SD	8	0.4702	0.2015
MB	19	0.435	0.2419
BR	22	0.313	0.4121
IB	22	0.4883	0.1823
WB	30	0.3764	0.318
HR	30	0.937	0.0002
TV	31	0.8698	0.0023
MO	39	0.9197	0.0004
FSR	49	0.8179	0.0071

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Figure 1. Corn seed & planter dust images

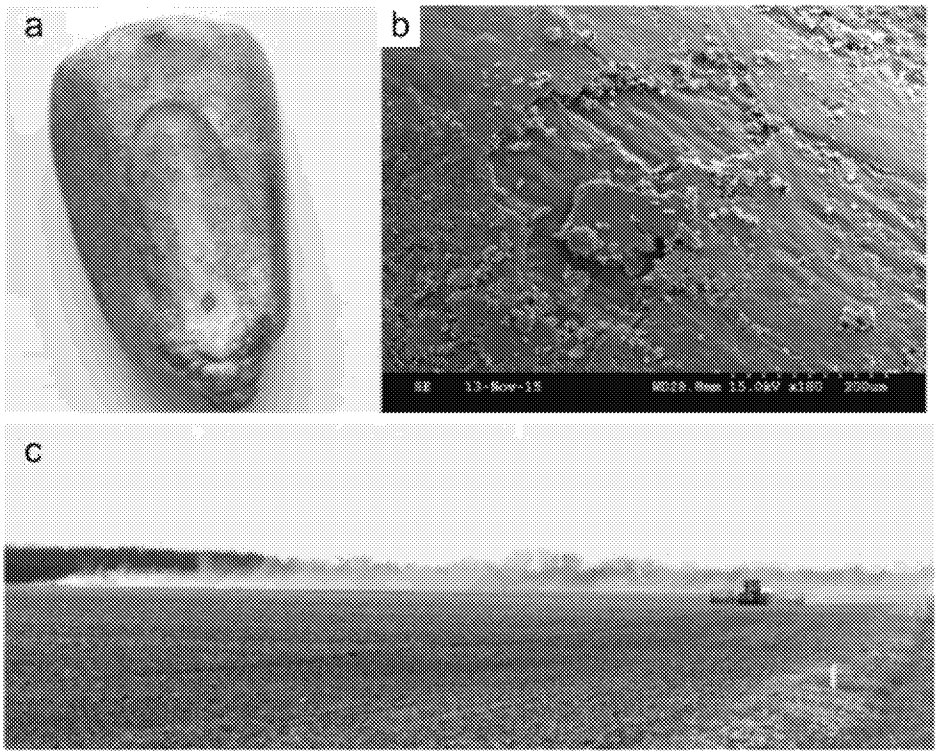

Figure 1. Seed treatments are applied to seeds as flowable solids that dry to form a coating. In corn, this coating results in visibly patchy coverage of the seed (a). The seed treatment forms particles of varying size on the surface of the seed as captured using scanning electron microscopy (SEM) (b). The striated surface visible in the center of the micrograph is the seed surface, uncovered by the broken seed treatment coating. Fragmented particles of the seed treatment coating are then emitted as planter dust during the sowing process (c). Macrophotography was performed by M. Spring, and SEM preparation by K. Kaszas.

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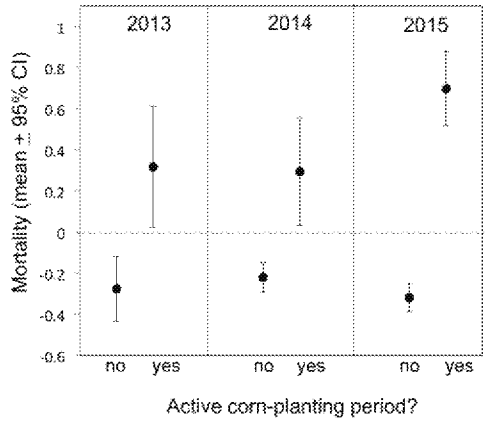
Figure 2. Three year data of mortality, clo+thi in pollen, and planting

Figure 2. Associations between honey bee worker mortality, clothianidine and thiamethoxam residues in bee-collected pollen, and corn-planting. (a) Honey bee worker mortality (per hive) during planting and non-planting periods. Dashed line represents the hypothetical value as if mortality is consistent across sampling periods. (b) Concentrations of clothianidine and thiamethoxam detected in pollen samples (per site) collected during planting and non-planting periods. (c) Honey bee mortality (site average)

(a)



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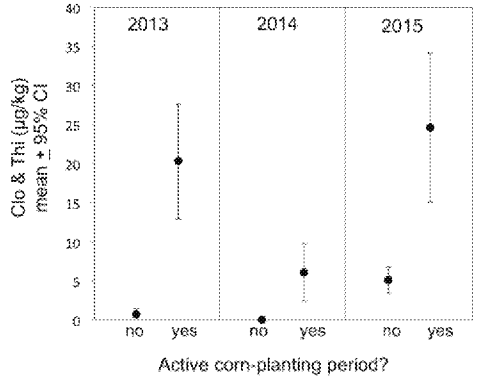

(c)

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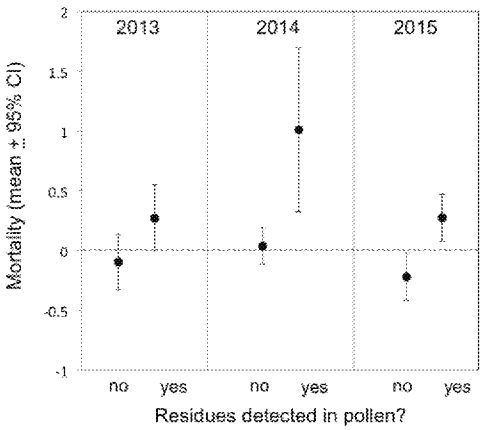
2013
2014
2015
Mortality (mean +.. 95% CI)
2
1.5
1
0.5
0
-0.5
-1
no
yes
no
yes
no
yes
Residues detected in pollen?

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Figure 3. Clo + thi in bee breads before, during, and post planting.

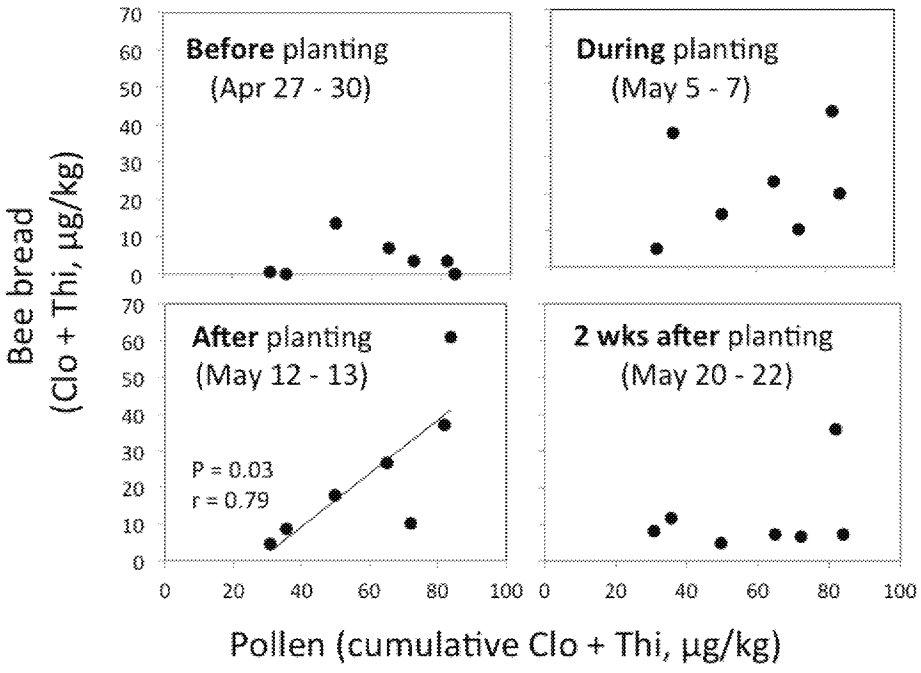

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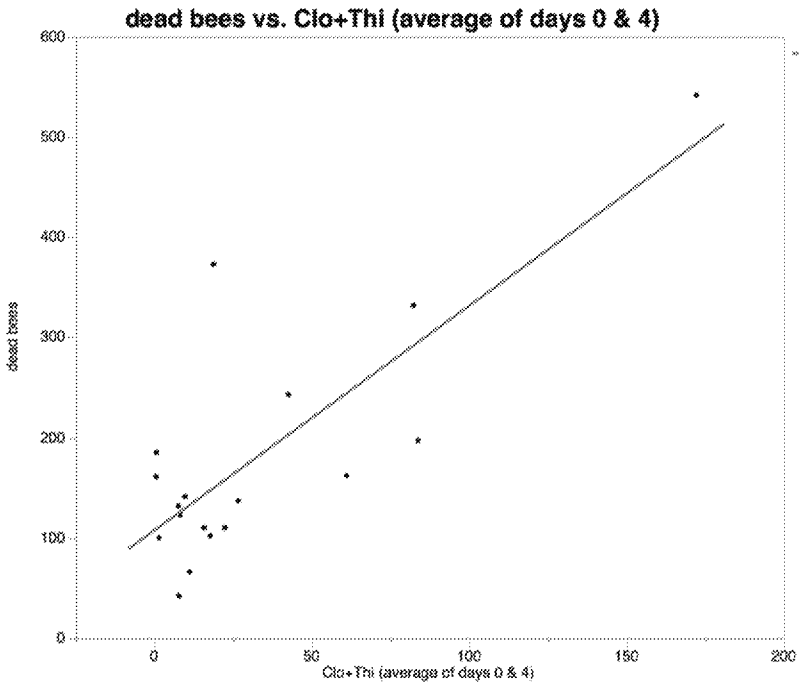
Figure 4. closed nucs worker mortality and clo+thi concentration (ng/g)



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